Tumor suppressors: **Linking cell polarity and growth control**

Andreas Wodarz

The *Drosophila* tumor suppressor genes *scribble*, *discs large* and *lethal giant larvae* appear to act in a common pathway. Mutations in any of these genes lead to loss of apical–basal cell polarity and overproliferation of epithelia, revealing a close connection between cytoarchitecture and growth control.

Address: Institut für Genetik, Heinrich-Heine-Universität Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany.
E-mail: wodarz@uni-duesseldorf.de

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Mutations in tumor suppressor genes are found in most human tumors and are believed to be a prerequisite for the accumulation of additional mutations that lead to uncontrolled growth and metastasis of cancer cells. The gene products encoded by tumor suppressor genes fall into different categories, including cell surface receptors such as Patched, cell adhesion molecules such as E-cadherin, cytoplasmic proteins such as PTEN and APC, and nuclear factors such as p53 and the retinoblastoma protein (Rb). Most of the known tumor suppressor gene products are involved in signal transduction pathways that lead either to cell-cycle arrest or apoptosis. Consequently, mutations in these genes cause overproliferation and survival of cells that otherwise would have been eliminated by cell death.

In *Drosophila*, several tumor suppressor genes have been identified as sites of mutations that cause overgrowth of imaginal disc epithelia. The mutants can be divided into two main categories: hyperplastic mutants, where overgrowth occurs without loss of epithelial structure; and neoplastic mutants, where hyperproliferation is accompanied by loss of apical–basal polarity and abnormal cell shapes. Until recently, the latter group comprised only two genes, *discs large* (*dlg*) and *lethal giant larvae* (*lgl*) [1,2]. They have now been joined by a third gene with very similar properties, *scribble* (*scrib*) [3,4].

The consequences of mutations in the *scrib* gene have been studied in the embryonic epidermis [3], in the follicular epithelium that surrounds the germ cells during oogenesis, and in imaginal disc epithelia [4]. In *scrib* mutants, severe defects in epithelial organization and polarity were observed in all three tissues. In the follicular epithelium, *scrib* mutant cells round up, become multilayered and invade in between germ cells. Imaginal discs of *scrib* mutants display dramatic overgrowth and can reach cell numbers that are five-fold higher than wild-type discs. Epithelial organization and apical–basal polarity is completely lost in these discs, demonstrating that *scrib* is a novel neoplastic tumor suppressor gene [4]. The phenotypes of *dlg* and *lgl* mutants in the follicular epithelium [5,6] and in imaginal discs [7,8] are virtually indistinguishable from those of *scrib* mutants, which prompted Bilder *et al.* [4] to investigate the potential functional connection between these three genes.

In addition to their similar mutant phenotypes, the three tumor suppressor genes show strong genetic interactions. Embryos lacking only the zygotic expression of any one of the three genes develop normally until overproliferation of imaginal discs begins at late larval stages. In contrast, zygotic double mutants of *scrib* and *dlg*, or of *scrib* and *lgl*, are embryonic lethal, which points to a dose-dependent interaction of their respective gene products [4]. Further evidence for a close link between *scrib*, *lgl* and *dlg* comes from immunolocalization studies. The three proteins colocalize at the basal–lateral membrane of epithelial cells. Scrib and Dlg are highly concentrated in the region where the septate junction forms (Figure 1), while Lgl shows a more widespread distribution and is not exclusively membrane-associated. This localization is disrupted in mutants for any of the three genes, indicating that Scrib, Lgl and Dlg are dependent on each other for their correct cellular localization [4].

The *scrib* gene was initially found in a screen for mutations affecting apical–basal polarity in embryonic epithelia [3]. Mutant *scrib* embryos show severe disorganization of the epidermis, accompanied by the failure to form a zonula adherens. The zonula adherens is a belt-like structure around the apex of epithelial cells (Figure 1), which is essential for maintenance of the epithelial tissue organization. The main structural components of the zonula adherens are homophilic cell adhesion molecules of the cadherin family that are connected to the actin cytoskeleton via catenins, cytoplasmic proteins that bind to the intracellular domain of cadherins. In *scrib*, *lgl* and *dlg* mutants, components of the zonula adherens are mislocalized, indicating that all three genes are required for assembly of the zonula adherens [4]. But how can Scrib, Lgl and Dlg affect formation of the zonula adherens if they are not components of the zonula adherens and localize to a more basal position at the lateral membrane?

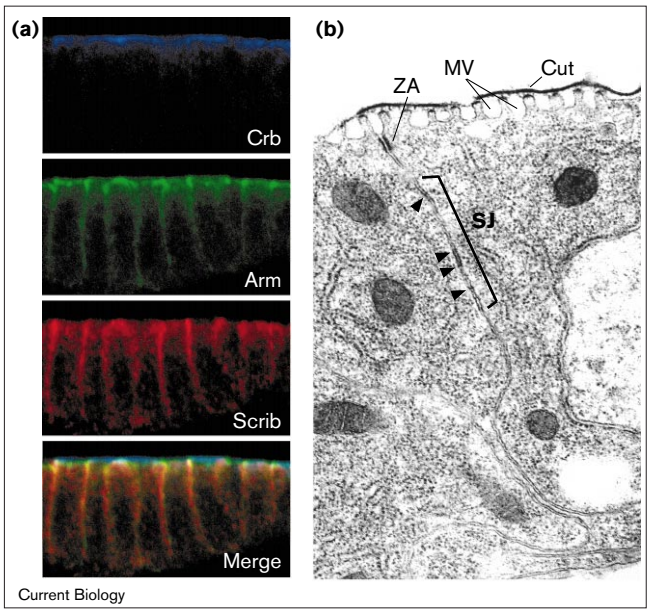
Mutants in the gene *crumbs* (*crb*) also fail to form a zonula adherens and show loss of epithelial polarity [9,10]. Crb is a component of the apical plasma membrane and is highly concentrated just apically of the zonula adherens (Figure 1).

Overexpression of Crb causes an expansion of the apical membrane domain at the expense of the basal–lateral domain and also leads to disruption of the zonula adherens [9,11,12]. In *scrib*, *lgl* and *dlg* mutant embryos, Crb is found at ectopic positions along the basal–lateral membrane [3,4], which could explain why zonula adherens assembly is perturbed in these mutants.

These observations support a model in which positioning of the zonula adherens depends on distinct sets of proteins localized apically, as well as basally, of the zonula adherens components themselves (Figure 1). How can Crb and the three tumor suppressor proteins cooperate to position the zonula adherens? Crb is a large transmembrane protein that binds via its cytoplasmic tail to Discs Lost (Dlt), a multi-PDZ domain protein [12,13]. Although no additional binding partners of Dlt have been identified so far, it is likely that Crb and Dlt assemble a multiprotein complex in the apical cytocortex that may prevent movement of zonula adherens components into the apical membrane domain.

A similar scenario can be envisioned for Scrib and Dlg, as both contain multiple protein–protein interaction domains (Figure 2). Dlg binds to a variety of transmembrane and cortical proteins, and is required for the formation of septate junctions. Thus, it is quite possible that Dlg and Scrib are also components of a large protein complex that may set the basal margin of the zonula adherens. According to this model, the plasma membrane is gradually divided into discrete subdomains that have a defined order along the apical–basal axis and do not intermingle. How these membrane domains are specified, and how they are stabilized, remains to be shown, but it is likely that the proteins discussed above are involved in this process.

Figure 1



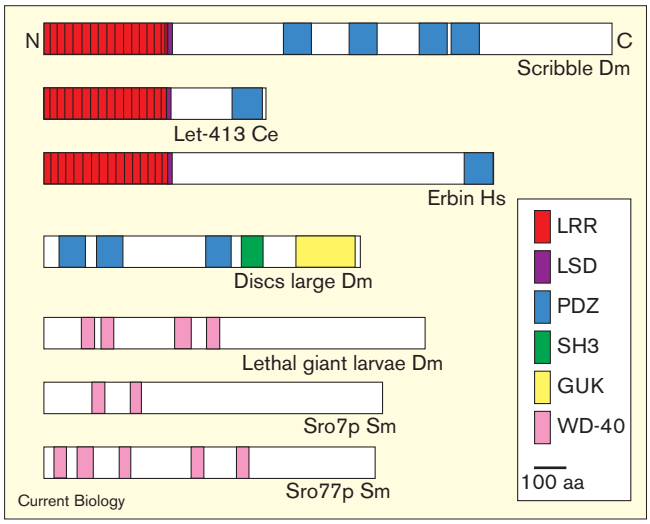
Subcellular localization of polarity determinants in the embryonic epidermis of *Drosophila*. **(a)** Shortly after gastrulation (stage 8), Crb, Armadillo (Arm, the *Drosophila* β-catenin homolog) and Scrib are localized in distinct subdomains of the plasma membrane. Crb is localized on the free apical surface of the epidermis; Arm is predominantly localized to the region where the zonula adherens will form; and Scrib is localized more basally in the region of the future septate junction. **(b)** Transmission electron microscopic image of the epidermis of a stage 16 *Drosophila* embryo. At this stage, epidermal cells are fully polarized and secrete cuticle (Cut) that is attached to the tips of apical microvilli (MV). The zonula adherens (ZA) is positioned just below the apical surface and the septate junction (SJ) forms basally of the zonula adherens. Individual septae are marked by arrowheads. Apical is up in all panels.

Clues to the role of Lgl in the control of cell polarity have come from studies of Lgl homologs in yeast. These homologs, Sro7p and Sro77p (Figure 2), are involved in exocytosis and bind to the plasma membrane SNARE protein Sec9p [14]. SNAREs are receptors found on the inner face of the plasma membrane (tSNAREs) or on the surface of secretory vesicles (vSNAREs), and are required for fusion of vesicles with the plasma membrane. Sro7p can functionally compensate for loss of Sro77p, and vice versa. However, *sro7Δ sro77Δ* double mutants show a dramatic accumulation of secretory vesicles in the cytoplasm, suggesting that the vesicles are unable to fuse with the plasma membrane.

In both yeast cells and vertebrate epithelial cells, vesicle fusion occurs in specific subdomains of the plasma membrane. In yeast, a large protein complex assembles at the tip of the emerging bud and forms a targeting patch that specifies the site for vesicle docking. In vertebrate epithelial cells, homologs of the yeast proteins Sec6p and Sec8p localize to the basal–lateral membrane in the vicinity of adherens and tight junctions. Antibody-blocking experiments have shown that the Sec6–Sec8 complex is essential for plasma membrane delivery of basal–lateral proteins, but is not required for apical transport [15]. Thus, the loss of cell polarity in *lgl* mutants could be explained by a defect in exocytosis of basal–lateral proteins, which is consistent with the expansion of apical markers observed in the mutants.

Two recent papers [16,17] have provided additional evidence for a general role of Scrib-related proteins [18] in the control of cell polarity and zonula adherens assembly. The *Caenorhabditis elegans* protein Let-413 (Figure 2) is localized at the basal–lateral membrane of embryonic epithelia and is required for the assembly of the zonula adherens [16], similar to Scrib in *Drosophila*. The human protein ERBIN (Figure 2) is also present at the basal–lateral membrane of epithelia and is responsible for correct localization of the ERBB2/HER2 receptor [17]. A similar dependence on PDZ domain proteins for basal–lateral localization has been described for Let-23, the

Figure 2



Domain structure of *Drosophila* tumor suppressor gene products and of some related proteins from other species. Protein motifs were identified using the SMART server (http://smart.embl-heidelberg.de/). Abbreviations: LRR, leucine-rich repeat; LSD, LAP-specific domain; PDZ, PSD-95, Dlg, ZO-1; SH3, Src homology region 3; GUK, guanylate kinase; WD-40, WD-40 repeat; Dm, *Drosophila melanogaster*; Ce, *Caenorhabditis elegans*; Hs, *Homo sapiens*; Sm, *Saccharomyces cerevisiae*.

C. elegans homolog of the ERBB2/HER2 receptor [19]. Interestingly, basal–lateral localization is essential for the full signaling capacity of Let-23.

Together, these data clearly establish a central role for the tumor suppressors Scrib, Lgl and Dlg in the control of cell polarity. However, the big question remains as to why these genes are also required for the prevention of uncontrolled growth. One attractive possibility would be that overproliferation results from mislocalization of signaling proteins, for instance growth factor receptors or β-catenin. Many signaling proteins have been identified as proto-oncogenes and mislocalization may lead to their inappropriate activation. Furthermore, the failure to form a zonula adherens in the tumor suppressor mutants could impinge on contact inhibition, a poorly understood mechanism that prevents uncontrolled proliferation. Additional work will be required to figure out why cell polarity and growth control are so intimately connected.

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